

Comparison of modified Thrombelastograph and Plateletworks whole blood assays to optical platelet aggregation for monitoring reversal of clopidogrel inhibition in elective surgery patients

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Clinically monitoring recovery from clopidogrel and nonsteroidal anti-inflammatory drug (NSAID) inhibition requires whole blood assays corresponding to a standard methodology such as platelet-rich plasma aggregation monitored optically (OPA). We compared OPA, using an ED₅₀ dose of adenosine diphosphate activation, with 2 whole blood assays, Plateletworks (PWA) and modified Thrombelastograph (TEG). Two sets of assays were performed on 43 surgery patients while on clopidogrel and off clopidogrel to determine the reversal of absolute and relative inhibition. The modified TEG had Spearman correlations with OPA for absolute ($\rho = .424$; $P = .006$) and relative inhibition ($\rho = .742$; $P < .0001$). PWA correlations with OPA gave absolute ($\rho = .28$; $P = .08$) and relative inhibition ($\rho = .46$; $P = .004$) values. Bland-Altman analysis indicated agreement of both tests with OPA, showing constant biases of about 18% and some dependency on mean magnitude error. Cohen effect size thresholds defined nonresponders as $< 7.7\%$ clopidogrel inhibition relative to baseline recovery of full platelet function. Apparent nonresponse to clopidogrel or lack of platelet recovery did not correlate with statin or NSAID therapies. These PWA and modified TEG whole blood assays could prove useful for monitoring the reversal of clopidogrel and NSAID inhibition before surgery. More important, these assays done at baseline and after beginning clopidogrel therapy could monitor the effectiveness for the individual patients with cardiovascular disease and help identify the need for alternative therapies. (J Lab Clin Med 2005;145:309-15)

Abbreviations: AA = arachidonic acid; ADP = adenosine diphosphate; CV = coefficient of variation; KH = kaolin and heparinase; MA = maximum amplitude; NSAID = nonsteroidal anti-inflammatory drug; OPA = optical platelet aggregation; PRP = platelet-rich plasma; PPP = platelet-poor plasma; PWA = Plateletworks assay; TEG = Thrombelastograph

The widespread use of nonsteroidal anti-inflammatory drugs (NSAIDs) and clopidogrel, with the attendant risk of bleeding during surgical procedures,^{1,2} has increased the need for point-of-care whole

blood assays correlated with a standard methodology, such as optically monitored platelet aggregation (OPA). OPA was used during the development of clopidogrel^{3,4} but not in later clinical studies, because of

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expertise, time, and equipment constraints. In studies where OPA has been done, baseline responses to adenosine diphosphate (ADP) have large standard deviations. These range from $\pm 9\%$ for a selected group of 25 patients with hyperresponsive platelets⁵ to up to $\pm 18\%$ for 92 randomly selected patients receiving stents.⁶ Platelet inhibition by clopidogrel has been calculated both as absolute^{7,8} and percent changes^{9–13} from this variable baseline platelet response. In some studies, an arbitrary $< 10\%$ inhibition was used to indicate nonresponders to clopidogrel therapy.^{8,10–12}

Whole blood assays for monitoring clopidogrel inhibition include flow cytometry,^{5–7,11} platelet function analyzer (PFA-100; Dade Behring, Deerfield, Ill),^{5,14} Ichor/Plateletworks analyzer (PWA) (Helena Laboratories, Beaumont, Tex),⁸ and whole blood impedance aggregation.^{5,11} Both platelet function analysis and whole blood impedance assays proved not as sensitive as OPA to clopidogrel inhibition. ADP-induced platelet activation monitored by flow cytometry was sensitive to clopidogrel, but this assay also was not directly correlated with OPA.⁵ PWA strongly correlated with OPA for monitoring ADP-induced platelet aggregation^{15,16} but did not agree with OPA concerning impairment of platelet function after cardiopulmonary bypass.¹⁷

We tested a modified Thrombelastograph (TEG) Haemostasis Analyzer (model 5000; Haemoscope, Niles, Ill) heparin-anticoagulated whole blood assay.¹⁸ This assay monitors activator-dependent platelet–fibrin interaction, correlates with OPA, and is sensitive to both clopidogrel and NSAID inhibition.¹⁸ In this study we compared the sensitivity/specificity of PWA and modified TEG whole blood assays to OPA for the detection of clopidogrel and NSAID inhibition and recovery of platelet function in elective surgery patients.

METHODS

Our institutional review board, acting under Helsinki Guidelines, reviewed the protocol used to obtain informed consent from 43 elective surgery patients. Enrolled patients were on a standard 75 mg/day clopidogrel therapy (with and without NSAID) for > 30 days for previously diagnosed cardiovascular disease. They were initially assayed while on clopidogrel therapy and then assayed off clopidogrel and NSAIDs for an average of 11 days, to allow platelets to recover normal function. Patients were excluded if they had thrombocytopenia, diagnosed hemophilia, or chronic bleeding problems.

Three different assays were carried out: PWA using citrate-anticoagulated blood (1:9 ratio of 100 mmol sodium citrate to whole blood); TEG using blood anticoagulated with 14.7 U/mL sodium heparin; and OPA using a Chrono-Log Aggregometer (Chrono-Log, Havertown, Pa) and heparin-anticoag-

ulated blood to isolate platelet-rich plasma (PRP) and platelet-poor plasma (PPP). Blood was drawn into Vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and assayed within 1 hour for whole blood assays and 2 hours for OPA responses to 1 μmol ADP (Chrono-Log), made up as 100-fold concentrated stock solution.

OPA assays were carried out for 10 minutes after the addition of 1 μmol ADP, and the maximum percent aggregation was determined as described previously.¹⁹ Heparin significantly potentiates platelet aggregation with low-dose ADP.²⁰ In our previous studies with normal subjects, 1 μmol ADP elicited close to an ED_{50} aggregation response ($37\% \pm 5\%$ vs a maximum $68\% \pm 13\%$ aggregation with 100 μmol ADP) using heparinized PRP and was found to be sensitive to both clopidogrel and NSAID inhibition for both OPA and modified TEG.¹⁸ We used heparin, allowing the same ADP dose and assayed without platelet count adjustment, to make OPA more comparable with the whole blood assays. This also allowed us to complete OPA assays within 2 hours. The 3 assays were each evaluated with respect to patients' platelet counts, either at baseline or after discontinuation of clopidogrel. Linear regression r^2 values were all $< .004$, and Spearman P values were all $> .26$, indicating no significant influence of platelet counts, which ranged from 99,000 to 427,000/ μL (mean, 180,000/ μL), to the response to 1 μmol ADP.

The reproducibility of the assay was evaluated with a normal volunteer on 4 separate days both before and during clopidogrel therapy. The baseline percent OPA gave a mean of $52\% \pm 5\%$. During days 3–7, on a clopidogrel dose of 75 mg/day, the mean was $25\% \pm 5\%$. The coefficients of variation (CV) were .092 and .204, respectively.

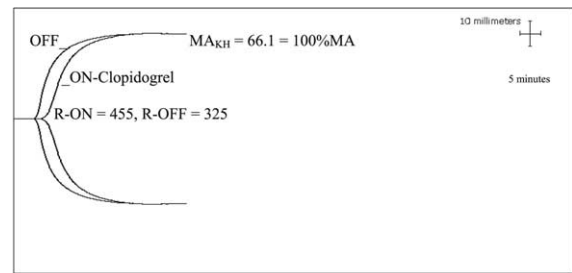
The PWA assay for percent aggregation uses a single-platelet counting Coulter technology before and after exposure to ADP to calculate aggregation, as described previously.^{15,16} In this study it was modified from the manufacturer's recommendations to make it comparable to OPA, continuously stirred at 37°C. The PWA used citrate-anticoagulated blood instead of heparin in accordance with the manufacturer's recommendations, to avoid platelet microaggregate formation.²¹ Blood was added in 1-mL aliquots to plastic tubes containing a stir bar, prewarmed to 37°C for 5–10 minutes, and placed in a 37°C thermostated tube holder and stirred at 1000 RPM. After 1 minute, 1 μmol ADP was added, and after another 30 seconds, the tube was placed in the instrument for single-platelet counting. A stirred sample, without added ADP, was used for the baseline platelet count. The percent aggregation was calculated as the percent reduction of baseline single platelet counts by added platelet activator. In repeat assays ($n = 8$) of a single sample drawn from a normal donor, the platelet counts of unstirred blood (average, $244,000 \pm 14,000/\mu\text{L}$) were not significantly different ($P = .212$; paired t -test) after stirring ($237,000 \pm 8000$). However, the percent aggregations obtained following the manufacturer's recommendations for mixing at room temperature ($78\% \pm 5\%$) versus our conditions ($85\% \pm 1\%$) were significantly different ($P = .013$; paired t -test). Our methodology also improved the CV from .065 to .016.

The modified TEG assay has been described previously.¹⁸ Heparin-anticoagulated whole blood is clotted by a reptilase-Factor XIIIa activator mixture (Haemoscope). This activator requires physiological calcium levels to function, thus precluding the use of citrated blood. The maximum amplitude (MA) is proportionate to platelet activation. Responses were compared to the MA of the same blood clotted with kaolin and heparinase (MA_{KH}) following the manufacturer's instructions. A percent MA response (modified TEG %MA) was calculated by subtracting the MA without platelet activation (MA_0) from the MA with ADP activation, dividing by MA_{KH} minus the MA_0 , then multiplying by 100%. This is analogous to the calculation done to determine OPA percent aggregation. The reproducibility of this assay was evaluated with a normal volunteer as described earlier for OPA. The baseline %MA responses assayed on 4 successive days had a mean of $56\% \pm 4\%$, which dropped to $6\% \pm 4\%$ after 3–7 days on 75 mg/day clopidogrel. The CVs were .076 for baseline and 1.238 on clopidogrel.

Of the 3 assays, the modified TEG is slightly sensitive to hematocrit. The affect of hematocrit is to lower MA_0 at high hematocrit levels, presumably by interfering with fibrin network shear viscosity. The linear regression was $r^2 = .172$ with a slope of $-.58$ mm/percent hematocrit and a Spearman correlation $P < .0001$. Hematocrit similarly lowered MA_{KH} , with $r^2 = .228$ with a slope of $-.72$ mm/percent hematocrit and a Spearman correlation $P < .0001$. However, there was no significant correlation of hematocrit with the %MA response to ADP (Spearman correlation $P = .628$).

Statistics. Absolute inhibition values are the off-clopidogrel minus the on-clopidogrel platelet responses. Relative percent inhibition is the absolute inhibition divided by the off-clopidogrel platelet responses, with the result multiplied by 100%. Paired *t*-tests were used to establish significant changes from individuals' baseline responses. Significance of group differences was tested by independent-sample *t*-tests for normally distributed data and by Mann-Whitney *U*-tests for data that followed other distributions. The χ^2 test was used for categorical data. Strength of linear association was evaluated using both linear regression and Spearman correlation. The OPA results were used to determine clopidogrel responder versus nonresponder status of the patient. The inhibition effects of clopidogrel on ADP-induced OPA in this study was evaluated using Cohen effect size cutoffs²² to define inhibition effects. This uses the absolute differences between baseline and posttreatment responses of the study population to define nonresponse as a $< .2$ fraction of the $\pm 16.6\%$ standard deviation of the absolute difference between baseline and posttreatment OPA. Our nonresponders were defined as $< 3.3\%$ absolute inhibition. Dividing the absolute changes defined by the Cohen size effect by the baseline average OPA value of 42.6% corresponds to $< 7.7\%$ for nonresponder OPA inhibition. Bland-Altman bias plots of the differences between the whole blood assays and OPA versus the means of the 2 assays for each set of data, as well as linear regression analysis of these data, were used to check for proportional, magnitude, or systematic biases. These were generated by the Analyse-It + Clinical Laboratory 1.65 pro-

A. Standard Kaolin/Heparinase TEG Traces of Patient Sample ON- and OFF-Clopidogrel



B. Modified TEG Assay of Patient Response to 1 μ M ADP, ON- and OFF-Clopidogrel

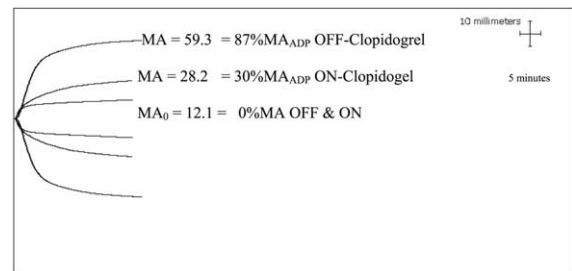


Fig 1. Standard (a) and modified (b) TEG traces of a patient on and off clopidogrel. The vertical line indicates MA scale; the crossing horizontal line indicates 5 minutes time. The MA and corresponding %MA for each trace are indicated in the figure. For this individual, we calculate a percent inhibition by clopidogrel of 66% in terms of MA.

gram (Analyse-It Software, Leeds, UK). All other statistical tests were done with StatView 4.0 software (SAS Institute, Cary, NC).

RESULTS

In standard TEG assays, platelet function is detected in the MA of the thrombin-formed fibrin network resulting from thrombin activation of platelet–fibrin interactions. This thrombin activation is stronger than any other platelet activator; thus the effects of thromboxane A_2 and ADP receptor antagonists are observed only as a slightly delayed reaction time (*R*) to clot formation, as shown in Figure 1. Comparing the surgery patients on and off clopidogrel, we observed a significant shortening of *R* values after discontinuation of antiplatelet therapies ($P = .0002$; paired *t*-test), decreasing from an average of 320 ± 121 to 247 ± 107 seconds. However, neither the change in *R* on and off clopidogrel nor the absolute *R* values on clopidogrel correlate by Spearman analysis with either absolute or relative percent inhibition changes detected by OPA ($\rho < .05$; $P > .7$). There was no significant change in MA_{KH} on and off clopidogrel (mean, 67 ± 6 and 67 ± 5 , respectively; $P = .749$; paired *t*-test) or angle (65 ± 10 and 69 ± 9 degrees, respectively; $P = .09$; paired *t*-test).

Table I. Platelet assay percent responses with 1 μ mol ADP, off and on clopidogrel, for OPA, modified TEG, and PWA

	Mean	SD	SE	Median	Range	CV	Skew	Kurtosis
OPA								
Off clopidogrel	43	18	3	46	4–80	0.42	–0.22	–0.74
On clopidogrel	29	14	2	29	0–65	0.48	0.34	–0.01
Absolute inhibition	14	17	3	10	–9–50	1.23	0.58	–0.62
Relative inhibition	31	27	4	30	–13–100	0.88	0.30	–0.55
Modified TEG								
Off clopidogrel	48	29	4	54	0–102	0.61	–0.07	–1.17
On clopidogrel	28	17	3	25	4–66	0.63	0.60	–0.78
Absolute inhibition	20	22	3	12	–22–76	1.19	0.61	–0.35
Relative inhibition	48	37	6	51	–10–100	0.78	0.01	–1.38
PWA								
Off clopidogrel	70	19	3	73	18–100	0.28	–0.70	–0.15
On clopidogrel	37	31	5	31	0–96	0.84	0.48	–1.13
Absolute inhibition	33	30	5	29	–13–99	0.93	0.59	–0.46
Relative inhibition	49	38	6	52	–5–100	0.77	–0.12	–1.45

SD = standard deviation; SE = standard error; CV = coefficient of variation.

Using the modified TEG assay, in the absence of thrombin generation, platelet ADP activation is observed as an increased MA (Fig 1). The calculated ADP %MA response is comparable to OPA and sensitive to antiplatelet drugs such as aspirin or clopidogrel.¹⁸ In these assays ADP-activated TEG values for *R* (on clopidogrel, 41 \pm 27 seconds; off clopidogrel, 38 \pm 18 seconds; $P = .476$; paired *t*-test) and angle (on clopidogrel, 56 \pm 13 degrees; off clopidogrel, 54 \pm 13 degrees; $P = .472$) only reflect the efficiency of clot formation by Haemoscope's activator mixture.

A statistical analysis of the patients' platelet responses to 1 μ mol ADP on and off clopidogrel with and without NSAID therapy for the 3 assays is given in Table I. All 3 assays detected significant clopidogrel inhibition ($P < .0001$; paired *t*-test). We calculated clopidogrel inhibition of the %MA both as relative percents and absolute values. All assays have a normal distribution and occasionally a higher response on clopidogrel, indicating negative inhibition. In a previous study using both clopidogrel-inhibited and clopidogrel-uninhibited platelets and various ADP doses to compare OPA and TEG, linear regression gave $r^2 = .65$.¹⁸ In the current study, with a smaller number of subjects and more limited activation conditions, modified TEG %MA was correlated by linear regression analysis with OPA for absolute ($r^2 = .15$) and relative percent inhibition ($r^2 = .55$). Similar relationships of PWA absolute and relative inhibitions to OPA gave r^2 values of .07 and .16, respectively. Spearman correlation evaluated the agreement between the assay methods. Modified TEG %MA correlated with OPA inhibition measurements giving absolute ($\rho = .424$; $P = .006$) and relative inhibition ($\rho = .742$; $P < .0001$) values. PWA

correlated with OPA with Spearman values for absolute ($\rho = .28$; $P = .08$) and relative inhibition ($\rho = .46$; $P = .004$).

By linear regression analysis, absolute inhibition by clopidogrel has a positive relationship ($r^2 = .389$; $P < .0001$) to the baseline OPA response of the patients [Fig 2(a)]. The OPA baseline response of the < 7.7% inhibition nonresponders (37% \pm 21%) versus responders \geq 7.7% (45% \pm 17%) was somewhat lower (but not significantly so) by analysis ($P = .22$; unpaired *t*-test). The modified TEG [Fig 2(b)] and PWA [Fig 2(c)] also gave positive slopes ($r^2 = .122$ and .071, respectively; $P = .022$ and .099, respectively) with respect to baseline reactivity, in agreement with OPA.

In view of the higher correlation of relative inhibition values, these were used to compare the agreement of the 2 whole blood assays to OPA. Bland-Altman bias plots for the assays relative to OPA are shown in Figure 3. Both assays demonstrate good agreement within \pm 1.96 standard deviations. Both have similar constant test biases, about 18%. There was no indication of significant proportional error, but there was evidence of some dependency on magnitude error. Linear regression analysis of the data to determine the linearity and strength of this magnitude error gave slopes of .36 and .48, r^2 values of .17 and .11, and P values of .003 and .019 for the modified TEG and PWA assays, respectively.

Table II summarizes the patient group characteristics and compares apparent clopidogrel responders to nonresponders in this study. Atorvastatin has been suggested to antagonize clopidogrel inhibition,⁸ but we did not observe this. NSAIDs act synergistically with clopidogrel,⁷ and the patients in this study were instructed

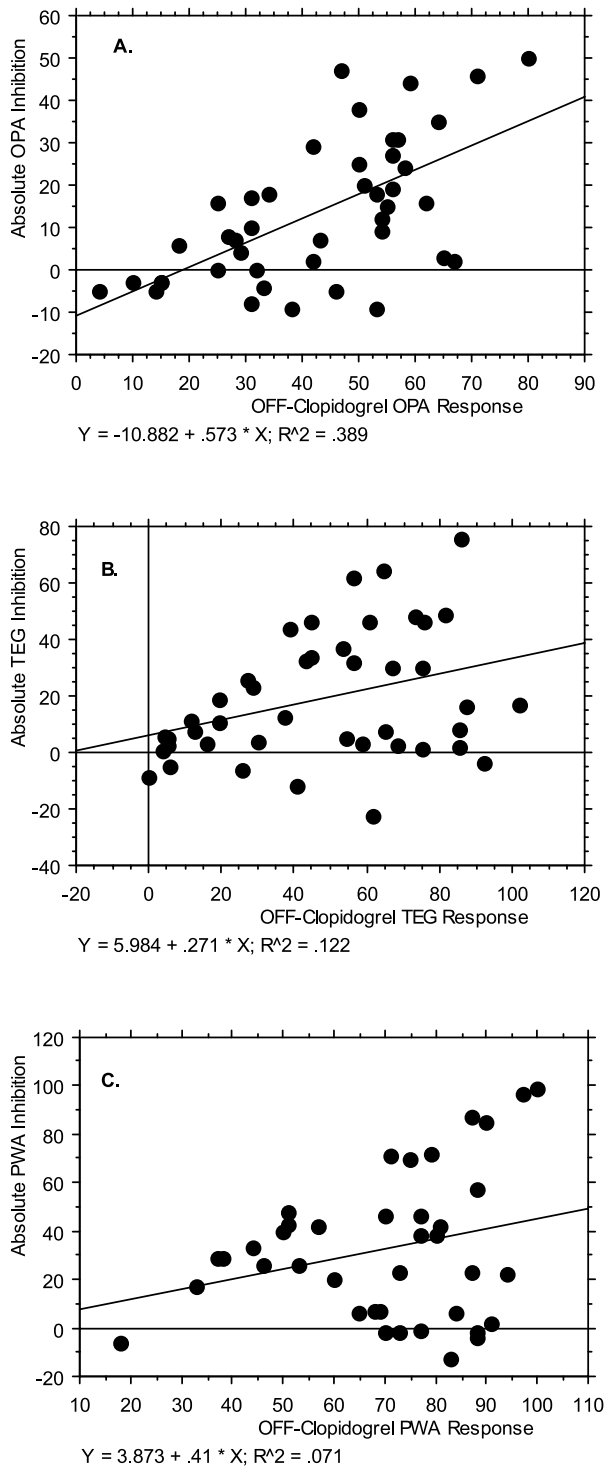
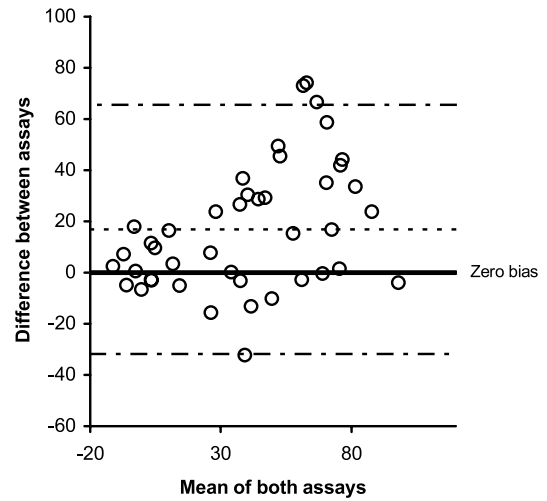


Fig 2. Linear regression correlation of combined patients' absolute clopidogrel inhibition with baseline platelet responses for (a) OPA, (b) modified TEG, and (c) PWA. Formulas for fitted lines and r^2 values are shown.

A. Modified TEG Versus OPA Relative Inhibition



B. PWA Versus OPA Relative Inhibition

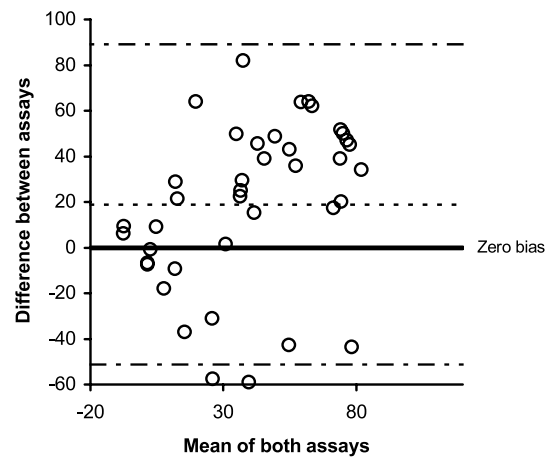


Fig 3. Bland-Altman bias plots for (a) modified TEG and (b) PWA compared with OPA. The plots show the differences in the measured relative inhibition between the 2 assays plotted against the mean relative inhibition of the 2 assays for each patient. The dashed upper and lower lines indicate ± 1.96 standard deviations of the average of the 2 assays, the solid trend line indicates the zero bias between methods, and the dotted line indicates the actual mean systematic bias (19% for PWA and 17% for modified TEG).

to discontinue both therapies. Although NSAIDs could contribute slightly to ADP inhibition,¹⁸ there was no significant difference in NSAID history between apparent responders and nonresponders. The residual effects of clopidogrel therapy, similar to aspirin therapy, should be eliminated by platelet replacement over an 8- to 12-day period.²³ Shorter periods off clopidogrel could result in smaller differences between the on- and

Table II. Patient group characteristics, clopidogrel responders versus nonresponders (< 7.7% OPA relative inhibition)

Clinical variable	Elective surgery group (n = 43)	
	Clopidogrel responder (n = 32)	Clopidogrel nonresponder (26%, n = 11)
Mean age (range)	65 (45–85)	65 (44–87)
Males	63%	55%
Atorvastatin	19%	0%
Baseline NSAID	59%	64%
Mean days off clopidogrel (range)	11 (2–30)	11 (2–27)

All differences are nonsignificant ($P > .05$, by the Mann-Whitney U -test, age and days, or the χ^2 test).

off-clopidogrel responses and less apparent inhibition. However, there was no significant difference in the period of on and off days (ranging from 2 to 30 days) for nonresponders (< 7.7% OPA relative inhibition) versus responders ($P > .85$; Mann-Whitney U -test). Neither was there a significant Spearman correlation of percent OPA inhibition with the number of days after discontinuing antiplatelet therapy ($\rho = .05$; $P = .72$). In this study we did not attempt to determine noncompliance in this patient group, and some patients may have continued taking antiplatelet drugs, accounting for the lack of recovery of platelet function.

DISCUSSION

Two different whole blood assays to monitor platelet function were used in this study. The modified TEG assay allows formation of a fibrin network in the absence of thrombin with an increased MA dependent on platelet activators similar to OPA. Under these activation conditions, it has a similar distribution of responses close to the midpoint of the range of possible responses. It differs from OPA by assaying whole blood with only very gentle mixing and monitoring platelet interaction with a fibrin network instead of a fibrinogen monomer. In contrast to TEG and OPA, PWA provides a sensitive single-point evaluation of platelet aggregation in whole blood by single-platelet counting. In this study we introduced some modifications to make the test more comparable to the other assays and to decrease variability due to mixing and temperature differences.

Despite differences in the 3 tests, there is a significant Spearman correlation among them. Bland-Altman analysis indicates correspondence between the whole blood tests and OPA with a slight constant difference and some dependency on magnitude error. This magnitude error suggests that both whole blood tests overestimate

recovery from clopidogrel inhibition relative to OPA at higher mean levels of inhibition. This could be due to a test error or to greater observed clopidogrel inhibition in a whole blood environment.

Previous studies^{6,7} found an inverse relationship of absolute inhibition by clopidogrel with the baseline responsiveness to ADP, suggesting that the higher responders would be less inhibited by clopidogrel and have a higher percentage of nonresponders. Clopidogrel inhibition can be overcome by higher doses of ADP,^{10,18} and resistance to inhibition can be overcome with higher doses of clopidogrel or longer treatment times.⁷ In this study both whole blood assays agreed with OPA that higher baseline responses to 1 μmol ADP showed greater absolute inhibition. Depending on the assay technique and ADP doses, individuals with more ADP-responsive platelets assayed with a high dose of ADP might not show clopidogrel inhibition. However, assays in which mean baseline responses are very low will underestimate inhibition. Thus it is important to select a dose of ADP for a particular assay that is reasonably close to the baseline ED_{50} for the population of subjects.

Evaluating treatment effectiveness is problematic with quite variable baseline and posttreatment responses. This problem was addressed by Cohen,²² who related treatment effectiveness to an individual's change from baseline relative to the standard deviation of observed changes in the entire treated population. This approach standardizes the cutoffs of effectiveness to the inherent variability of each measurement technology. With some refinements, the Cohen size effect threshold remains an accepted standard in the medical literature.²⁴ We hope that this approach of comparison with a standard assay methodology, such as OPA, can help standardize results from different studies and assay technologies.

Because no clinical endpoints were measured in this study, we cannot say what extent of platelet function recovery is important in preventing bleeding complications. It should be noted that some individuals had greater platelet responses to ADP while on clopidogrel as opposed to baseline, as has been noted by others.⁶ This could reflect a relatively small inhibition by clopidogrel and the influence of other uncontrolled variables affecting platelet function.

Speculations. Both PWA and modified TEG whole blood assays correlate with OPA for detecting clopidogrel inhibition. Because both PWA and modified TEG use whole blood without the need for centrifugation, both qualify as point-of-care tests for rapidly monitoring clopidogrel therapy in a clinical setting. These assays can be used to evaluate the significance of differences in inhibition by clopidogrel or other platelet

inhibitors and recovery of platelet function before surgery on patient outcomes. Moreover, such assays might allow the development of a database to guide the clinician as to the safety of proceeding with given surgical procedures or monitoring emergency care patients on platelet inhibitor therapies. Perhaps most important for the individual patient being treated for cardiovascular disease or thrombotic stroke is the possible availability of a clinical assay to monitor the effectiveness of clopidogrel therapy.

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REFERENCES

1. Hongo RH, Ley J, Dick SE, Yee RR. The effect of clopidogrel in combination with aspirin when given before coronary artery bypass grafting. *J Am Coll Cardiol* 2002;40:231–7.
2. Pothula S, Sanchala VT, Nagappala B, Inchiosa MA Jr. The effect of preoperative antiplatelet/anticoagulant prophylaxis on postoperative blood loss in cardiac surgery. *Anesth Analg* 2004;98:4–10.
3. Caplain H, Cariou R. Long-term activity of clopidogrel: a three-month appraisal in healthy volunteers. *Semin Thromb Hemost* 1999;25:21–4.
4. Thebault JJ, Kieffer G, Lowe GD, Nimmo WS, Cariou R. Repeated-dose pharmacodynamics of clopidogrel in healthy subjects. *Semin Thromb Hemost* 1999;25:9–14.
5. Serebruany VL, Malinin AI, Jerome SD, Lowry DR, Morgan AW, Sane DC, et al. Effects of clopidogrel and aspirin combination versus aspirin alone on platelet aggregation and major receptor expression in patients with heart failure: the Plavix Use for Treatment Of Congestive Heart Failure (PLUTO-CHF) trial. *Am Heart J* 2003;146:713–20.
6. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003;107:2908–13.
7. Payne DA, Hayes PD, Jones CI, Belham P, Naylor AR, Goodall AH. Combined therapy with clopidogrel and aspirin significantly increases the bleeding time through a synergistic antiplatelet action. *J Vasc Surg* 2002;35:1204–9.
8. Lau WC, Gurbel PA, Watkins PB, Neer CJ, Hopp AS, Carville DG, et al. Contribution of hepatic cytochrome P450 3A4 metabolic activity to the phenomenon of clopidogrel resistance. *Circulation* 2004;109:166–71.
9. Izaguirre-Avila R, De la Pena-Diaz A, Barinagarrementeria-Aldatz F, Gonzalez-Pacheco H, Ramirez-Gutierrez AE, Ruiz-Sandoval JL, et al. Effect of clopidogrel on platelet aggregation and plasma concentration of fibrinogen in subjects with cerebral or coronary atherosclerotic disease. *Clin Appl Thromb Hemost* 2002;8:169–77.
10. Jaremo P, Lindahl TL, Fransson SG, Richter A. Individual variations of platelet inhibition after loading doses of clopidogrel. *J Intern Med* 2002;252:233–8.
11. Gurbel PA, Malinin AI, Callahan KP, Serebruany VL, O'Connor CM. Effect of loading with clopidogrel at the time of coronary stenting on platelet aggregation and glycoprotein IIb/IIIa expression and platelet-leukocyte aggregate formation. *Am J Cardiol* 2002;90:312–5.
12. Muller I, Besta F, Schulz C, Massberg S, Schonig A, Gawaz M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost* 2003;89:783–7.
13. Muller I, Besta F, Schulz C, Li Z, Massberg S, Gawaz M. Effects of statins on platelet inhibition by a high loading dose of clopidogrel. *Circulation* 2003;108:2195–7.
14. Van der Planken MG, Claeys MJ, Vertessen FJ, Dilling D, Bosmans JM, Berneman ZN, et al. Comparison of turbidimetric aggregation and in vitro bleeding time (PFA-100) for monitoring the platelet inhibitory profile of antiplatelet agents in patients undergoing stent implantation. *Thromb Res* 2003;111:159–64.
15. Carville DG, Schleckser PA, Guyer KE, Corsello M, Walsh MM. Whole blood platelet function assay on the ICHOR point-of-care hematology analyzer. *J Extra-Corpor Technol* 1998;30:171–7.
16. Lau WC, Walker CT, Ogilby D, Walsh MM, Carville DGM, Guyer KE, et al. Evaluation of a BED-SIDE platelet function assay: performance and clinical utility. *Ann Cardiac Anaesth* 2002;5:33–42.
17. Menys VC, Belcher PR, Noble MI, Evans RD, Drossos GE, Pillai R, et al. Macroaggregation of platelets in plasma, as distinct from microaggregation in whole blood (and plasma), as determined using optical aggregometry and platelet counting, respectively, is specifically impaired following cardiopulmonary bypass in man. *Thromb Haemost* 1994;72:511–8.
18. Craft RM, Chavez JJ, Bresee SJ, Wortham DC, Cohen E, Carroll RC. A novel modification of the Thrombelastograph assay, isolating platelet function, correlated with optical platelet aggregation. *J Lab Clin Med* 2004;143:301–9.
19. Born GVR. Quantitative investigation into the aggregation of blood platelets. *J Physiol* 1962;16:67–8.
20. Mascelli MA, Kleiman NS, Marciniak SJ Jr, Damaraju L, Weisman HF, Jordan RE. Therapeutic heparin concentrations augment platelet reactivity: implications for the pharmacologic assessment of the glycoprotein IIb/IIIa antagonist abciximab. *Am Heart J* 2000;139:696–703.
21. Ault KA, Rinder HM, Mitchell JG, Rinder CS, Lambrew CT, Hillman RS. Correlated measurement of platelet release and aggregation in whole blood. *Cytometry* 1989;10:448–55.
22. Cohen J. *Statistical power analysis for the behavioral sciences*. rev ed. New York: Academic Press; 1977.
23. Lins R, Broekhuysen J, Necciarri J, Deroubaix X. Pharmacokinetic profile of 14C-labeled clopidogrel. *Semin Thromb Hemost* 1999;25:29–33.
24. Middel B, van Sonderen E. Statistical significant change versus relevant or important change in (quasi) experimental design: some conceptual and methodological problems in estimating magnitude of intervention-related change in health services research. *Int J Integr Care* 2002;2:1–22.